GROWTH AND DIFFERENTIATION OF THE COLONIES OF *ZOOTHAMNIUM ALTERNANS* (CLAP. AND LACHM.)

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Introduction

In a preceding publication (1922) I have insisted on the fact that colonial Vorticellidæ constitute an intermediary step between a population of like cells (cultures of free Infusoria) and a multicellular organism; unlike free cells with unlimited power of division, whose population growth theoretically follows a geometrical progression. The colonies of *Epistylis,* of *Carchesium,* or of *Zoothamnium* generally have a limited growth, following a special cycle, independent of a possible sexual cycle. In these colonies the lineage of each cell is perfectly defined by dichotomous ramifications of a common peduncle, and it is possible to show in a large number of cases the existence of somewhat differential divisions giving two sister cells whose power of multiplication is different. In certain species (*Epistylis arenicolæ, Epistylis Perrieri*) the first divisions can be dichotomous and equal, so that the mass growth of a number of individuals follows a geometrical progression; but soon the sister cells resulting from each division multiply unequally, and the growth approaches more or less an arithmetical progression.

On the other hand, the study of the growth of the common peduncle, which is considered as a product of the protoplasmic activity, shows that the latter may decrease in course of time. But the Vorticellidæ colonies form, from time to time, migrating individuals which may be of the same size as the other individuals (*Carchesium, Epistylis*) or more voluminous (some *Epistylis,* some *Zoothamnium,* called heteromorphic). In these individuals, and in these only, appear secretory granules already observed by Engelmann and more recently (1926) by Wesenberg-Lund, which seem to be connected with the formation of the peduncle, and one can consider the hypothesis of an active substance, or of a transformable substance, produced in a definite quantity and periodically, by certain individuals, which is divided among the descendants of the latter and at the same time is diminished little by little.

It appears then that the growth of a group of cells may be limited by

factors somewhat internal but altogether independent of the hypothetical notion of a " factor of senescence " [1]

The *Zoothamnium* called heteromorphic, about which I have given some detail in my paper of 1922, seems to give the most typical examples as to the rôle of these internal, or properly cellular factors, in the general form of growth of a colony and its limitation.

Claparède and Lachmann described in 1858 a marine species, *Zoothamnium alternans* (described later by Möbius under the name of *Z. Cienkowskii*) ; the aspect of the colonies, they say, is that of " un arbre à branches courtes et très régulièrement alternantes. La forme de ces familles a sa cause dans un arrêt de division spontanée qui frappe en général l'un des deux individus issus de chaque division. Lorsqu'un individu *A* se divise en deux individus *B* et *B'*, l'un des deux, *B* par exemple, ne se forme qu'un pédoncule fort court et son développement reste stationnaire à partir de ce moment, tandis que l'autre, *B'*, secrète un pédoncule plus long, puis se divise en deux nouveaux individus, *C* et *C'*, dont le premier, qui est toujours du côté de la branche opposée à celui où se trouvait l'individu *B*, ne forme qu'un pédoncule très court et ne se divise pas davantage tandis que *C'* forme un pédoncule plus long et se divise en deux individus *D* et *D'* et ainsi de suite."

That is not all; in *Z. alternans* and in *Z. arbuscula* Ehrb. or *Z. geniculatum* Ayrton (see Wesenberg-Lund, 1925, and Furssenko, 1925) the migrating individuals which will be the origin of new colonies and will thus begin a new cycle, are distinguished not only by a few morphological characters, but also by their voluminous size and the well-determined place where they originate in the colony, generally at the junction of the main branches. These large migrating individuals are the " ciliospores " of Wesenberg-Lund or " macrozoides " of Furssenko, much larger than the " trophozoides " or " microzoides " which constitute the most numerous individuals of the colony.

Ehrenberg had observed these individuals in *Z. arbuscula*, and had noticed that they result from the growth of an individual not unlike the others, but always situated at the junction of a branch. This author admits that one of the two individuals issued from a bipartition on the branch while the other grows without dividing, thus being, he says " the aunt " of the individuals of the branch. Claparède and Lachmann find this same condition in *Z. alternans*, but sometimes this growing indi-

[1] In other publications (1925–26) I tried to show that in several very different cases the idea of a factor of senescence could be replaced either by the hypothesis of differing speeds in a group of transformations necessary to cellular activity, or by the assumption of a " probability " of transformation which would be too long to develop here. (See Fauré-Fremiet and Laura Kaufman, 1928, and Fauré-Fremiet and H. Garrault, 1928.)

vidual may undergo a division. *Zoothamnium alternans* (Claparède and Lachmann) is found frequently on the coasts of Brittany; I have found it in abundance in Woods Hole and was able to follow the different stages of the colony cycle and of the formation of the "ciliospores." I observed a few phenomena of conjugation, quite sporadic, but I have not observed a sexual cycle analogous to the one discovered by Wesenberg-Lund in *Z. geniculatum* or described by Furssenko in *Z. arbuscula.*

Technic

In order to follow the complete evolution in a large number of colonies, I have used numbered slides, ruled in squares with a diamond point. These slides were first placed in a crystallization dish containing numerous colonies of *Z. alternans.* After several hours, they were removed and placed in a Petri dish containing sea water and examined under a binocular microscope. All individuals recently attached were carefully located and designated in numeral order; those whose peduncle had already developed or had already given the first division were removed with a needle.

After this operation, the slides were placed vertically on frames floating in an aquarium through which ran a strong current of sea water; this was done to avoid the deposit of particles and of microörganisms. The slides were then examined periodically and the different stages of the development of each colony were carefully recorded in function of time.

When the cytological examination of a colony is necessary, it is always easy to detach this colony with a fine pipette, in order to study it under the high power, *in vivo,* or after fixation.

The best technic for the study of the nuclear apparatus is the fixation by OsO_4 for a short time followed by boracic carmine stain. The presence (generally in the Vorticellidæ) of a cuticle and the contractability of a peduncle constitute two technical difficulties which are not easy to overcome; it may be necessary to cut the colony with a fine scalpel in order to isolate certain individuals which it is necessary to fix and stain.

Structure of the Colonies

The appearance of colonies of *Z. alternans* is very nearly that of a palm (Fig. 1); they have a main trunk and oblique branches placed alternately in the same plane, on right and left of the axis; the main trunk always bears at the top a terminal individual of rather large size; the lateral oblique branches bear a variable number of small individuals;

finally along the trunk, at the juncture of the lateral branches, are found the voluminous migrating individuals either macrozooids or macrospores.

FIG. 1. A young colony of *Zoothamnium alternans* (Clap. and Lachm.), showing the main trunk and the alternate lateral branches. *TM*, terminal macrozooid; *Ci*, ciliospores at different stages of growth, located on the anterior side of the colony at the first division of each branch *D*, *E*, *G*, *H*. The branch *F*, in this case, bears, at the same place, only two microzooids apparently identical with the others.

The lateral branches of the colony observed in extension are almost always slightly curved in, and most of the individuals borne by these branches are inclined toward the outside of the curvature. The two sides of the palm are thus different, and one can define at the same time a base and a summit, an anterior and a posterior side.

The elements of symmetry of such a colony are a main axis represented by the trunk, and a median plane, antero-posterior, separating the two halves right and left.

As for all the other species of the genus *Zoothamnium*, the colonial peduncle bears an elastic tube whose rôle is passive, and a continuous "cordon central," dichotomically ramified, which represents the pro-

longation of the lower extremity of each individual; this central cordon has itself a protoplasmic tube (*I*) limited by a fine film and surrounding a muscular fiber which terminates at the basal part of each individual by a conical group of myonemes.[2] The migrating individuals, or "ciliospores," when liberated swim rapidly with their posterior ciliary crown. They are large individuals, flattened in the antero-posterior direction, and look like a top. They attach themselves by means of the scopula (*I*) and begin to secrete the peduncle. At the same time they lose their posterior ciliary crown and progressively take on again the ordinary subconical form.

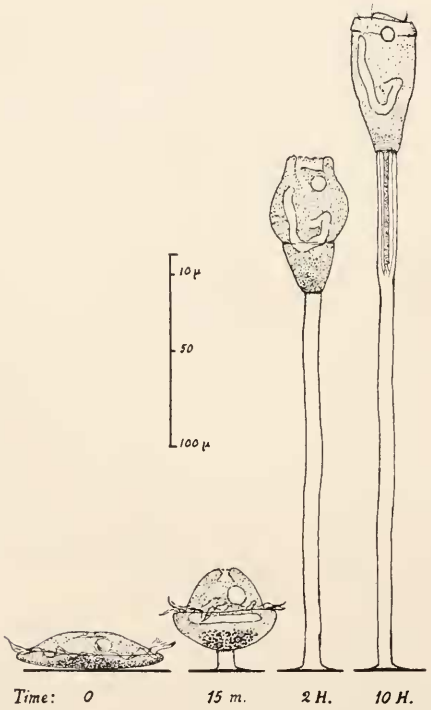


FIG. 2. Fixation of the ciliospore and construction of the peduncle. At first the top-like ciliospore turns quickly on the slide, then the building of the peduncle begins; the same individual is shown fifteen minutes after fixation. The ciliary crown slows down and disappears while the peduncle grows (Epistylis stage) during a short time (two hours); finally, one can see the differentiation of the "cordon central" and the muscular fiber (ten hours).

The peduncle is at first a solid cylindrical body of a fibrillar structure which grows rapidly ("Epistylis stage"); after two hours it reaches

[2] For the structure of the Vorticellidæ in general, and of the peduncle in particular, see Fauré-Fremiet (1906).

a length of about 250 μ. The secretion then begins to slow down and a section of the peduncle is ring-like; there is a central canal, at the bottom of which remains attached a part of the body of the infusorian, which from now on will lengthen itself along with the tube of the peduncle and become differentiated in a central cordon with the muscular fiber or "spasmoneme" (Fig. 2).

Six or seven hours (at the temperature of 21° C.) after the start of the secretion of the peduncle, the original individual undergoes a first unequal division which gives a macrozooid and a microzooid; the plane passing through these two zooids and the common peduncle is the median plane of symmetry of the future colony. The large cell remains clearly axial after this first division and continues to form actively the principal peduncle of the colony. After four to seven hours it undergoes a second unequal division; the interval between the following divisions is longer, from ten to sixteen hours; but always during the growth of the colony the terminal individual is a macrozooid, each division of which separates a microzooid in the median plane of the colony. The successive series of terminal microzooids constitutes a main strain perfectly schematized by the axial trunk of the colony.

We shall designate each cell of this series by a Roman numeral representing the division which started it; we shall have then the original individual, or ciliospore, then the series of macrozooids, I, II, III, . . . X, etc.

We shall designate with capital letters the corresponding series of median microzooids detached from the main strain (microzooids of first order), *A, B, C,* . . . *J,* etc. Each branch of the colony is started by the division, alternately at the right and at the left of the median plane of each microzooid of the first order. But, in accordance with the diagram of Claparède and Lachmann, only one of the two cells resulting from such a division is the origin of a lateral limb; we shall designate it by a small letter preceded by the coefficient 1; the other cell remains median and will be designated by its capital letter preceded by the same coefficient 1.

At the beginning of the formation of the fifth branch, for example, we shall have first the division of the terminal macrozooid IV, which will give a new terminal macrozooid V and a median microzooid *E.* The latter will divide in a perpendicular plane to that of the division of IV, and will give two individuals, one of which, 1*E,* remains in the median plane while the other, 1*e,* situated for example at the right of this plane, will be the origin of the branch (Fig. 3).

Each branch has also a main axis and lateral branches but does not have a well-defined median plane nor median individuals. The division

of $1c$, for instance, gives rise to two cells apparently similar, $2e^1$ and $2e^2$. The individual $2e^1$ remains in the axis and gives at the new division $3e^1$ (axial) and $3e^2$ (lateral); $3e^1$ will give $4e^1$ (axial) and $4e^2$ (lateral), etc.

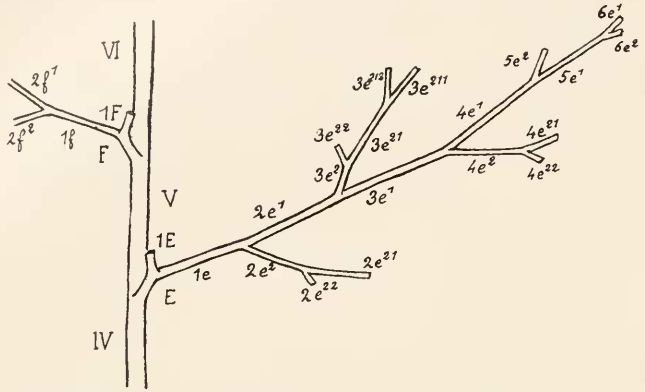


FIG. 3. Scheme of the branch E and the basis of the branch F, showing the lineage of the median microzooids $1E$ and $1F$ and the different microzooids.

Likewise the individuals $2e^2$, $3e^2$, and $4e^2$ will give successively two or three generations, the elements of which we shall designate by the symbols $2e^{21}$, $2e^{22}$, $3e^{21}$, $3e^{22}$, etc; according to the rule of Claparède and Lachmann $3e^{22}$ does not divide, but $3e^{21}$ gives $3e^{211}$ and $3e^{212}$; the number of generations formed by the lateral branches seems to be always rather limited.

The median individuals of the second generation: $1A$, $1B$, $1C$ . . . $1E$, etc., can divide once and give $1A^1$ and $1A^2$ for example. But while $1A$, $1B$, $1C$, and their two immediate descendants remain microzooids identical to these designated by the small letters, $1D$, $1E$ and the following ones, or the two cells of the second generation, $1D^1$, $1D^2$; $1E^1$, $1E^2$, etc., undergo a considerable growth and are transformed into ciliospores, or migrating macrozooids, which soon detach themselves from the common trunk to swim freely and to attach themselves later on.

It appears clearly then that during the growth of a colony of *Zoothamnium alternans* the two cells resulting from the division of one initial cell are never equivalent as to their "potentialities." But in confirming the observations of Ehrenberg and of Claparède and Lachmann, we may now make them more precise by showing that the progressive segregation of the power of multiplication and of the power of growth is very rigorously tied up with the respective position of the individual separated by the successive divisions. It seems then that a certain

number of divisions at least must be considered as differential divisions. The cytological examination confirms this interpretation.

First Division of the Initial Macrozooid

The first division is characterized, in a rigorously constant manner, by the unequal division of the macronucleus and of the protoplasm of the initial individual of the colony (Fig. 4) between the first two cells, the macrozooid *I* and the microzooid *A* (Fig. 5). A short time before this division, the macronucleus, which takes the shape of a long twisted rod, enlarges at one of its extremities in a compact mass. The other extremity is thin and often flattens slightly, and becomes elongated in the median plane of the individual. The two edges of this flat portion are often slightly thickened, so that a side view gives the impression of a structure in a horseshoe shape. The micronucleus remains near the thick extremity and soon lengthens into a spindle. Meanwhile the peristome and the scopula divide as well as the central cordon of the peduncle and soon an upper and a lower furrow, growing in depth toward each other, begin to separate two cells of very unequal size. The micronucleus completes its own division, then the macronucleus is divided unequally at the time when the two furrows join; the macrozooid (which remains the terminal individual on the axis of the colony) retains the thickened part of the macronucleus and a micronucleus; the microzooid (which becomes the first median individual *A*) retains the thin part of the macronucleus and a micronucleus (Fig. 6).

Considering the irregular shapes of the body and of the macronucleus in *Z. alternans*, it is impossible to calculate the corresponding volume and to establish the values of the nucleoplasmic relation. Nevertheless, it is clearly evident that the ratio N/P is greater in the microzooids than in the macrozooids, *i.e.*, the macronucleus is divided into two daughter cells even more unequally than the cytoplasm.

It is difficult to establish whether there exists a difference in composition between the two unequal extremities of the macronucleus divided between *I* and *A*. The "nuclear reaction" of Feulgen does not show any difference between these two parts, and their structure differs very little. Most frequently one can observe a linear orientation, in a continuous and parallel line of the chromatin granules (microsomes) in the thin part of the macronucleus which will be distributed by the division. On the other hand, the voluminous mass which remains in the macrozooid *I* shows an irregular distribution of its microsomes. This mass behaves as a chromatin reserve which would not be affected at all by the phenomena of division.

Supposing that the terminal condensation of the macronucleus repre-

sents a kind of segregation of the chromatin material, we shall describe this first unequal division as a differential quantitative and qualitative division.

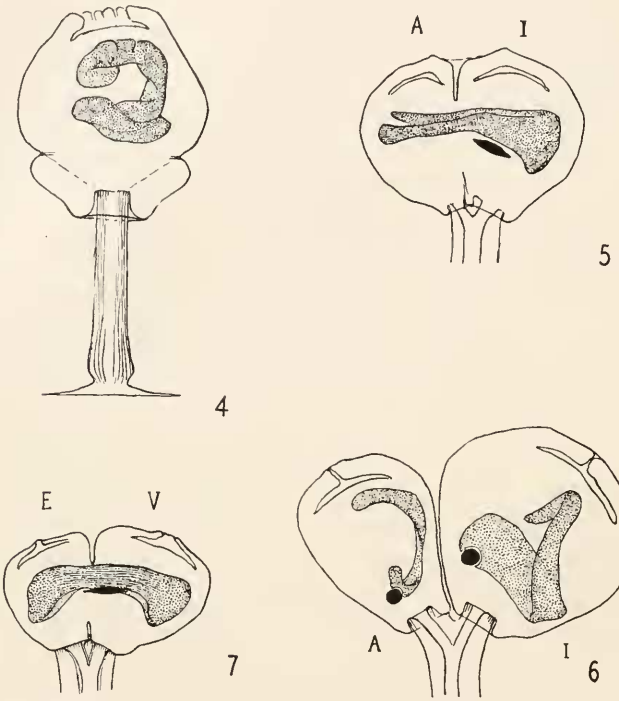


Fig. 4. Ciliospores at the beginning of the peduncle's formation, showing the appearance of the macronucleus before the first division.

Fig. 5. First cleavage of the ciliospore, giving the terminal macrozooid *I* and the median microzooid *A*. The figure shows the differential division of the macronucleus (figured by dotting) and the apparently equal division of the micronucleus (black—spindle stage).

Fig. 6. Later stage of the first cleavage, showing the terminal macrozooid *I* and the median microzooid *A;* macronucleus figured by dotting; resting micronucleus black.

Fig. 7. Fourth cleavage on the main strain giving the terminal macrozooid *V* and the median microzooid *E*. The qualitative equal division of the macronucleus (figured by dotting) is shown.

Later Divisions of the Individuals of the Main Strain

The division of the individuals *I* and *II* presents exactly the same differential character as that of the initial individual. It is different at the time of division of the individual *III*. In the latter, the macronucleus shows at the outset of the bipartition a symmetrical thickening at each of its granular extremities which appear entirely homologous.

The median part, finely striated, is divided, however, into two unequal parts by the division of the protoplasmic body, which isolates here again an axial and terminal macrozooid, V, and a median microzooid E (Fig. 7).

All the later divisions of the individuals from the main strain, *i.e.*, *IV, V, VI . . . X* etc., are of the same type, and we shall consider these divisions as quantitatively differential only.

Division of the Median Microzooids

The median microzooids, *A, B,* and *C,* which have received only the thin extremity of the initial macronucleus, undergo an almost equal division which gives for example $1A^1$ (median) and $1a^2$ (lateral) of the same dimension and of the same structure, both having a thin and twisted macronucleus, as well as the descendants of $1a^2$, $1b^2$, and $1c^2$ (Fig. 8).

On the other hand, the median microzooids, *D, E, F,* and the following undergo an unequal division, quantitatively and qualitatively differential, like that of the first three individuals: the ciliospores *I* and *II*. A short time before the division, when the median individual begins to lengthen in the transverse plane, its macronucleus takes the shape of an elliptic blade, presenting in a marginal point a large subspherical thickening. This thick part of the macronucleus, on the other hand, lengthens at the time of division and is divided between the two individuals 1*D,* 1*d,* 1*E* and 1*e,* etc. (Fig. 9).

These facts indicate that the differential division takes place at two different times from the fourth generation of the axial cells. For instance, when the division of *III* divides into *IV* and *D,* the microzooid *D* has a little less than a half macronucleus; but this half macronucleus is qualitatively similar to that of the macrozooid *IV*, having a granular terminal thickening. However, the microzooid *D* shows a nucleoplasmic relation, a ratio *N/P* superior to that of macrozooid *IV*, for the protoplasm has divided much more unequally than the macronucleus. It is a small individual with a large macronucleus.

When the microzooid *D* divides, the cytoplasmic division is almost equal, but the division of the macronucleus is qualitatively differential, because the thickened and granular part does not divide but goes whole to the median individual 1*D*. The outcome is that the ratio *N/P* is still increased in this individual.

The axial microzooids 1*D,* 1*E,* etc., can undergo a division and give for instance $1D^1$ and $1D^2$; but these two individuals, which remain median, soon begin to enlarge without dividing any further.

The microzooids 1*d*, 1*c*, etc., as said above, go through a series of divisions which always give individuals with long and slender macronuclei.

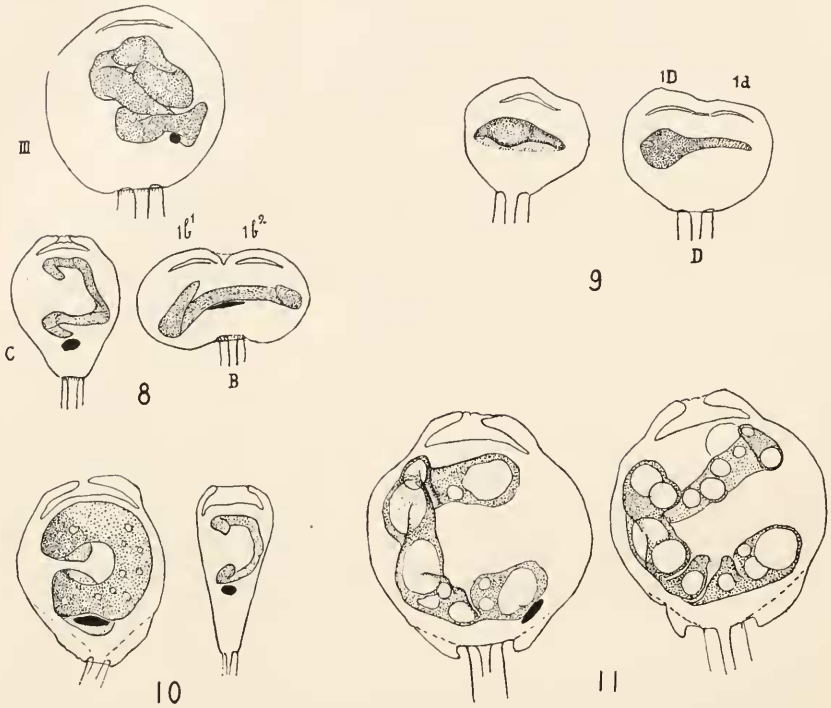


Fig. 8. Cleavage of the median microzooid *B*, giving, with equal division of the macronucleus, the microzooids $1b^1$ and $1b^2$. Comparison between the terminal macrozooid *III* and the median microzooid *C* (resting stage).

Fig. 9. Cleavage of the median microzooid *D*, giving the future median macrozooid 1*D* (ciliospore) and the microzooid 1*d*, with a differential division of the macronucleus.

Fig. 10. One median macrozooid (1*G* for example) at the beginning of its growth, and one microzooid of the corresponding branch. The large difference in size of the macronucleus is to be noted.

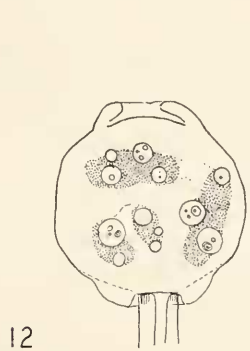
Fig. 11. Two median macrozooids during the time of growth. In the macronucleus, numerous large nucleoli are to be seen (figured as vesicles on the drawing).

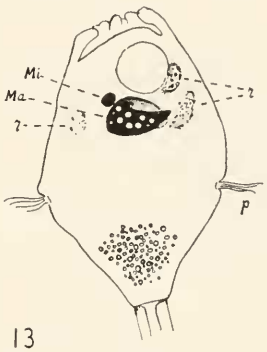
Growth of the Median Microzooids and Formation of the Ciliospores

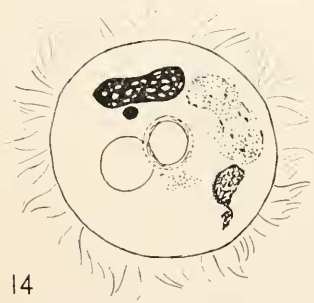
The median microzooids of the fourth generation (*D* or $1D^1$ and $1D^2$) and of the following generations (*E*, *F*, *G*, etc.) increase rapidly until they reach a length of about 55 μ to 70 μ, in one day, two days, or two and a half days.

The macronucleus, already voluminous, begins to grow and forms a very large horseshoe-shaped body. The micronucleus situated at the lower part in a slight depression lengthens into a spindle as in preparation for the division. While the macronucleus increases, rather refringent nucleoli appear in the midst of the chromatic granulations, not giving the reaction of Feulgen (Fig. 10).

Soon, while the protoplasmic growth goes on, it seems that the nuclear growth stops. The very numerous nucleoli alone still increase in volume (Fig. 11). Then the outline of the macronucleus disappears, the nucleoli project on the surface of the chromatic mass, and one can observe very numerous stages of disintegration and of degeneration of the macronucleus and of its fragments (Fig. 12).







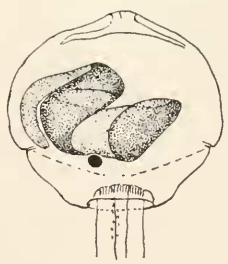


Fig. 12. Later stage of the median macrozooid's growth. Disintegration and disappearance of the macronucleus.

Fig. 13. One median macrozooid almost ready to leave the colony: *p*, posterior ciliary crown; *Ma* and *Mi*, macronucleus and micronucleus of the new nuclear apparatus; *r*, residual mass of chromatin.

Fig. 14. Top view of a median macrozooid (same stage as that shown in Fig. 12).

Fig. 15. Terminal macrozooid making the posterior ciliary crown and soon ready to leave the colony.

Finally, one sees in the center of the cytoplasmic mass containing a rather larger number of residual masses, a short macronucleus, arched, staining very intensely, containing only very small nucleoli, and accompanied by a resting spherical micronucleus (Figs. 13 and 14). This aspect, frequently observed, is that of a nuclear apparatus of new formation, and it is probable that the changes just described represent a phenomenon of endomixis. I was, however, unable to follow in the individuals stained *in toto* the fate of the spindle-shaped micronucleus observed in the preceding stages. It probably divides and makes up the new nuclear apparatus; but this stage was not observed in my set of preparations. At the end of the protoplasmic growth and when the nuclear changes are completed, a furrow appears around the median individual, at about the posterior third. It is the future ciliary crown, whose vibratile elements appear soon afterward. At the same time the organism flattens in the antero-posterior direction, and takes the shape of a top. The cytoplasm is filled with diverse inclusions, a great number of which are probably nuclear residue. In the posterior region, above the "scopula," appear very numerous inclusions which are not very refringent. Neutral red *in vivo* colors them a brownish red. These inclusions correspond to the secretion granules whose existence I have already mentioned in the migrating individuals of different Vorticellidæ.

There are still a few lipoid granules, and, toward the middle of the body, numerous small inclusions fixing neutral red in an intense red color. Iodine fixation gives a mahogany color, but the latter is not any stronger than for the microzooids.

The "ciliospore" which has thus been formed becomes almost lens-shaped. The peristome remains closed and the posterior ciliary fringe is animated with active movements which soon determine the liberation of the migrating individual (Fig. 15).

Growth of the Colonies of Zoothamnium alternans

At a temperature of 21° C., in an aquarium with running water, the growth of the colonies of *Z. alternans* goes on very regularly for a period of eight to ten days. Hence it is easy, by periodic examinations of a specific colony, to follow the increase in number of the individuals as a function of time. We have then a measure of the colony's growth. This measure is not very exact, because certain individuals grow without dividing and their mass is clearly larger than that of the others. However, the group of large cells given by the terminal macrozooid and the ciliospores is always rather restricted, and one can admit that the

appearance of the development is rather well represented by the variation in number of the individuals. A more important error may arise from the fact that some parasitic Infusoria (*Acineta*) very often get into the microzooids (especially the microzooid of the first branch) and multiply in this individual, which does not divide and soon falls off.

Because of this, it is necessary at every investigation to trace a total scheme of the colony studied, indicating the place of each individual, which with some practice, may be quickly made by examining the colonies in extension in a thin water layer with a low power objective. By this means it is possible to keep an account of the accidental influences; but when the number of individuals increases too much, beyond the eighth day, for example, this method of pointing becomes very difficult and soon impossible to use with precision.

TABLE I

Date	Time	Numbers of Colonies Examined											
		1	2	3	4	5	6	7	8	9	10	11	12
July 14	11 A.M.	0	0	0	0	0	0	0	0	0	0	0	0
" 15	11 A.M.	III	III	III	III	II	III	III	III	II	III	III	
" 16	12 M.	V	V	V	IV	IV	III	IV	IV	III	IV	IV	
" 17	4:30 P.M.	VII	VIII	VII	V	VI	V	VII	VI	V	VI		
" 18	9 A.M.	VIII	IX	IX	VII	VII	VII	IX	VIII		VI		
" 19	10 A.M.	IX	XII		VIII	VIII	VIII	XI	X				
" 20	11:30 A.M.	XI	XIV		XII	X	IX	XIII					
" 21	9 P.M.	XII	XVI		XIV			XV					
" 22	9 P.M.		XVII			XII							

Date	Time	Numbers of Colonies Examined											
		13	14	15	16	17	18	19	20	21	22	23	
July 14	11 A.M.	0	0	0	0	0	0	0	0	0	0	0	
" 15	11 A.M.	II	III	III		II	IV	I	III		II	III	
" 16	12 M.	IV	V	V		III	V		V		IV	IV	
" 17	4:30 P.M.	VI	VII			V	V		VII		VI	VI	
" 18	9 A.M.	VIII	VIII			VII			IX		VII		
" 19	10 A.M.												
" 20	11:30 A.M.												
" 21	9 P.M.												
" 22	9 P.M.												

The simultaneous study of the growth of the various colonies placed in apparently identical conditions, on the same slide or on adjacent slides, shows at first that the speed of growth is not the same for all the colonies. We have already seen that the interval between two divisions varies in rather large proportion, in the same stage, in two different colonies (*i.e.* four hours to seven hours between the division of *I* and that of *II;* ten hours to sixteen hours between the division of *II* and that of *III*).

Table I shows the records of twenty-three colonies (experiment commenced on the 14th of July); the figure 0 indicates the initial macrozooid at the beginning of the peduncle formation, and the Roman numbers indicate the number of the terminal individual on the main strain; we see, thus, that on the fourth day, there may be a difference of two generations between different colonies and that on the eighth day the difference may be four generations. The whole number of individuals borne by each colony differs, of course, proportionally.

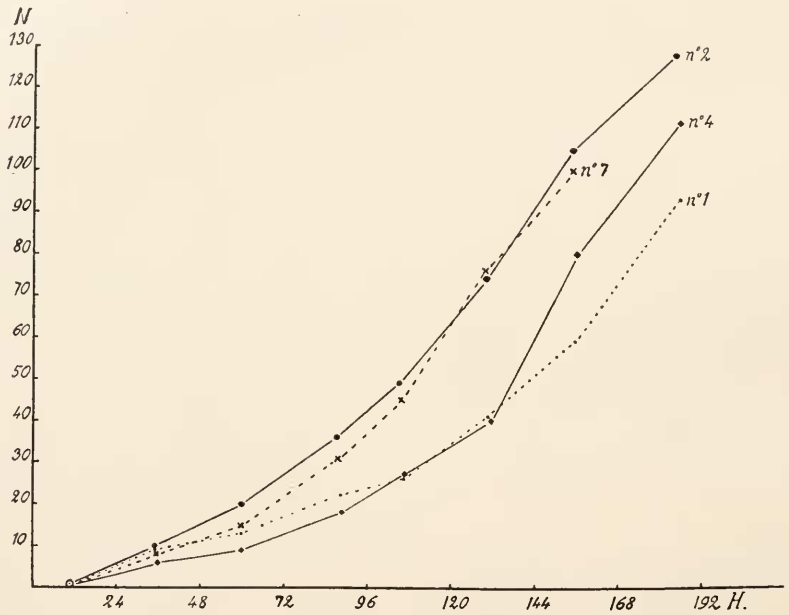


Fig. 16. Curves of growth from four colonies of *Z. alternans* (Nos. 1, 2, 4, and 7); number of the individuals in ordinates; time (in hours) in abscissæ.

The data relative to colonies Nos. 1, 2, 4, and 7 are plotted in the curve of Fig. 16. These are only gross numbers, there being no correction for some microzooids parasitised or dropped out. Besides, these various curves show that for each colony the rate of growth varies itself in the course of the growth; but it is difficult to determine the part of the accidental factors already mentioned and capable of introducing some disturbance.

Fig. 17 represents in function of time the genealogical and complete view of a colony having given sixteen generations on the main strain.

The essential data are given by the successive records of colony No. 2, completed, as regards the incomplete branches sprung from *A*, *B*, and *C*, by the data furnished by other colonies studied in the same experiment (3, 5, 20, etc.). Furthermore, the periods of some divisions have been settled according to the survey of the successive and periodical examinations of colony No. 2 with interpolations; I have kept account, in this case, of the interval settled with more precision than in other experiments in which either the first stages of the colony or the growth of a branch were connected at intervals of time most closely approached from hour to hour.

The curve represented in Fig. 18 is drawn according to this scheme. The daily increase of the number of individuals shows the following numbers:

Time (in hours)	Number of individuals	Increase of the unity of mass in 12 hours	Number of zooids made in 24 hours
0	1		1
12	2	2	
24	4	2	3
36	9	2.25	
48	15	1.66	11
60	23	1.58	
72	31	1.34	16
84	41	1.32	
96	55	1.34	24
108	66	1.20	
120	84	1.27	29
132	104	1.23	
144	122	1.17	38
156	137	1.12	
168	147	1.06	25

The first part of this tabulation shows a rather regular increase and such that the number of the individuals, *i.e.*, approximately the whole protoplasmic mass, doubles at regular intervals, from twelve hours to twelve hours.

Of course, we find again here, at first the geometrical progression of the ratio 2 which characterized the multiplication by bipartition of a mass of cells which keep always the same speed of growth. If we choose for unity of time this period of twelve hours, we see, however, that after the second day the rate of growth of the unity of mass, which averaged about 2, slows down progressively from 1.66 to 1.58 and 1.34, then persists for some time at a median and constant level: 1.32, 1.34, 1.20, 1.27.

Then, in a last period, this rate of growth again slows down with the

values 1.23, 1.17, 1.12, 1.06; but the difficulty in obtaining an exact enumeration does not permit a determination of its values when the colony approaches its greatest size.

Then it appears that the growth progressively slackens in the whole of the colony; *the time necessary to double the protoplasmic mass grows as the protoplasmic mass increases;* it is a limiting factor of the growth.

But it is evident that this factor (or limiting factor), in the case of *Z. alternans,* is not a factor of senescence which affects equally all the individuals, and involves a sort of progressive segregation, whose nuclear phenomena give a parallel objective picture.

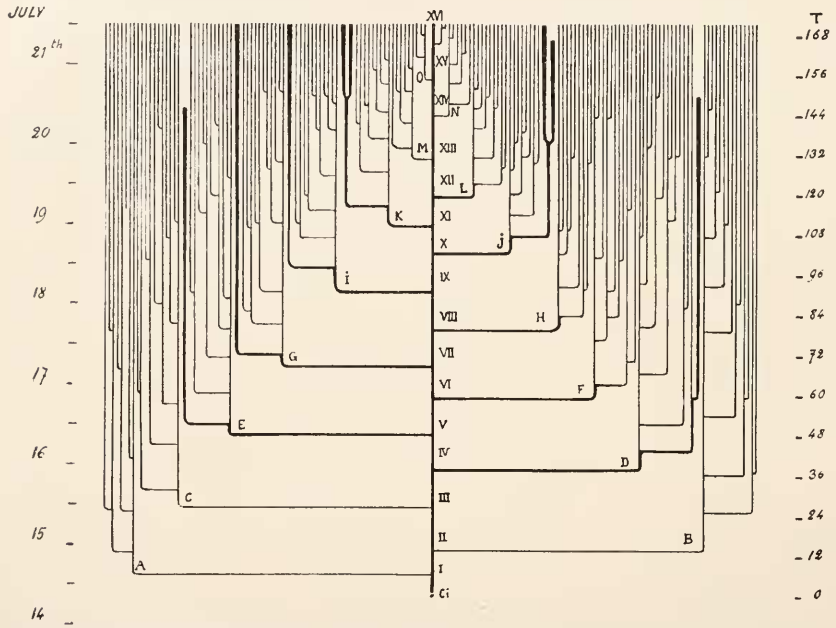


FIG. 17. Genealogical view of a colony at the sixteenth generation; time in abscissæ; lineage of each individual in ordinates.

This leads us to examine the case of the main strains. After the second division, which takes place rapidly, four to seven hours after the first one and at a temperature of 21° C., the rhythm of the bipartitions of the axial macrozooid slows down, (sixteen to seventeen hours between second and third divisions), then remains sensibly constant. During the entire growth of the colonies, more than twenty bipartitions of the axial macrozooid succeeded each other at intervals of ten to sixteen hours. The growth of the axial peduncle was fairly constant.

It seems then that during eight to ten days at least, the functional activity and the power of growth of the axial macrozooid remain constant, and, in the colonies already developed, one can observe the for-

nation of a posterior ciliary crown around the terminal individual. Thus the axial macrozooid can become a migrating individual equivalent to a ciliospore, but one never observes in this case the endomictic transformation of the nuclear apparatus. We have seen how the nuclear segregation which is established during the differential divisions seems to determine the characteristic features of the median individuals and of the microzooids. However, we must admit that the later divisions of the microzooids are still different, although they are not accompanied by a visible nuclear segregation.

According to the rule of Claparède and Lachmann, we can still distinguish in one branch one main strain and lateral strains.

The fourth branch, for instance, after the differential divisions which separate $1D$ and $1d$ may be represented as follows: $1d$ gives $2d^2$ and $2d^1$. Let us give the exponent 1 to the main strain of this branch; $2d^2$ gives $2d^{21}$ and $2d^{22}$ which do not divide any further; $2d^1$ on the contrary gives $3d^2$ and $3d^1$. The smaller branch issued from $3d^2$ has a principal axis, but the number of generations is reduced. The first division separates $3d^{22}$, which does not divide any further, and $3d^{21}$, which gives $3d^{212}$ and $3d^{211}$ without descendants. The individual $3d^1$ gives $4d^1$ and $4d^2$; $4d^2$ gives $4d^{22}$ without descendants and $4d^{21}$, which still gives $4d^{212}$ and $4d^{211}$ without descendants. The individual $4d^1$

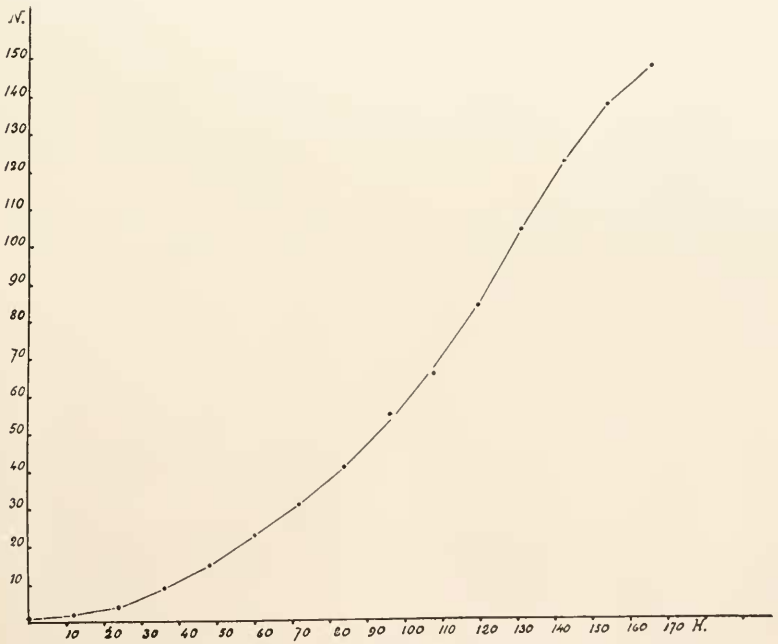


FIG. 18. Curve of growth of *Z. alternans* colony drawn from Fig. 17.

finally gives $5d^2$ without descendants, and $5d^1$ which divides into $6d^1$ and $6d^2$ without descendants.

The interval which separates the microzooid divisions is at first of the same order (or even more rapid) than the interval which separates the divisions of the axial individual; but it increases progressively and in such a colony, for example, the individuals of the sixth branch will represent six successive generations from the cell *F*, while its sister cell *VI* will have given during the same length of time ten successive generations.

We can see from Fig. 17, for instance, that the microzooids $2d^{22}$ and $2d^{21}$ live more than three days and a half without bipartition; such a fact is more typical with some microzooids of the earlier branches, *A* and *B*, which maintain themselves for more than five days without division. But after this time (corresponding to ten generations on the main strain of the branch) these individuals do not appear larger than the others; yet they feed and their protoplasm contains many digestive vacuoles. The decrease of the power of growth which characterizes these individuals is not dependent upon their age—and for this reason we cannot admit the notion of the factor of senescence—but of their position in the colony, as if the differential divisions assured the progressive segregation of a factor of growth. But we can still notice that this segregation, as it may be seen by the form of the growth of the branch *D*, for instance, is yet continued during the divisions of the microzooids which show no longer a differential appearance.

In short, if we bear in mind the main axis of the colony, its branches and its boughs, we see that the power of growth, and of multiplication, decreases according to a kind of gradient, in proportion with its removal from the main strain.

The differential character of the cellular divisions seems to be the essential condition which slows down and restrains the growth of the colonies of *Z. alternans*. But, theoretically at least, this restricted growth should go on indefinitely. It is not the case here. Secondary factors play here an important rôle; the development of different parasites (Protozoa, Protophytes) make it impossible to obtain a normal growth of the colonies beyond ten days, under ordinary laboratory conditions or in a natural marine environment; soon the last surviving individuals leave the common peduncle. The microzooids often form in this case a posterior ciliary wreath; their fate has not been determined.[3]

[3] A few cases of conjugation have been observed between a terminal macrozooid and a migrating microzooid. These cases were rare; the later phenomena were not followed.

Conclusions

The sexual cycle described by Furssenko and by Wesenberg-Lund in the voluminous species of *Zoothamnium* (*Z. arbuscula* Ehrb., *Z. geniculatum* Ayrton) is rather special and with *Z. alternans* (Clap. and Lach.) I have never observed anything similar, either on the Britanny coast or in my cultures at Woods Hole; I will not, then, attempt to compare the evolutionary cycle of these different species. The objective that has led me into the minute study of these colonies of Vorticellidæ is the cyclical evolution—generally considered—of an initial cell's lineage, which is here the foundation macrozooid or the "ciliospore."

The growth of colonies of *Z. alternans* is limited, in a great measure, by external agents such as parasitic infections, or the growth of animal and vegetable microörganisms which change the surrounding conditions of a specific colony.

In the cultures watched as described above, these various circumstances, somewhat accidental, are much reduced; yet the growth of each colony appears to be limited *in itself;* I have taken the common individual—the microzooid—as unity of mass, and I have observed that the rate of growth decreases in function of time for the whole of each colony studied; at the same time, some particular migrating individuals are formed and become the source of new colonies; it is precisely this "cyclical" appearance of growth in the colonies of Vorticellidæ that I have described in an earlier paper (Fauré-Fremiet, 1922); I have considered two different hypotheses: (1) the formation during the evolution of the migrating individuals of a limited stock of an hypothetical "active substance" which divides and becomes increasingly smaller with each generation of daughter-cells, or (2) a progressive modification of the intimate composition of the cells, variations which would be "corrected" only during the evolution of their own migrating cells.[4]

In any case, this cyclical and limited evolution gives to the colonies of Vorticellidæ (*Epistylis, Carchesium, Zoothamnium*) somewhat of an individualized character. In this regard, the case of *Z. alternans* is very striking. At first, the successive divisions of the cells derived from the first individual and the regularity with which they follow one another in exactly determinate planes which fix the general features of the colony, closely recall the process of a strictly predetermined cleavage, but one which would be complicated with a continuous growth.

Secondly, the existence in these colonies of a main strain and of secondary strains characterized by different nuclear qualities and different evolutionary properties recall in a certain measure the separation

[4] These suppositions have been examined and criticized in a very interesting work of G. Teissier (1928).

of the germinative and somatic strains during the cleavage of an *Ascaris* egg.

Thirdly and finally, we can characterize the individuality of the colony by the repartition of the power of growth and the power of multiplication of its cells according to a certain gradient.

In connection with another species of *Zoothamnium* Wesenberg-Lund also considers the notion of the individuality of the colony, for the various individuals are tied by the continuous protoplasmic thread of the ramified peduncle and this brings about in their mass rather a physiological unity. But the above-indicated characteristics are again met, more or less accentuated, in other colonial Vorticellidæ in the species *Epistylis* and *Carchesium*, for example, which do not show any protoplasmic connection between the zooids.

The case of these colonies is then nearer that of a " population " of cells, and their cyclical evolution appears very similar to populations of free Infusoria, studied by so many authors.

The case of *Z. alternans* is still, from this point of view, particularly interesting. In these species, the Claparède and Lachmann rule shows that two daughter-cells have not necessarily the same power of growth and of proliferation. I found the same rule (1922) in some species of the genera *Carchesium* and *Epistylis,* and more especially with *Epistylis arenicolæ* (n. sp).

Here there seemed to exist in the course of the successive bipartitions a kind of progressive segregation of the power of growth, but we find in *Z. alternans,* as an objective support of this hypothesis, the differential divisions, which are produced at the origin of each lateral branch and which indicate a kind of nuclear segregation.

In this species the main strain's cells which keep a constant nuclear appearance, keep also a constant rate of growth and, apparently, an indefinite multiplicative power. We witness, then, a cytological mechanism, probably independent of the external factors which rule the functional differentiation of the cells belonging to the same family, in a process of growth.

This cytological factor, or those which are superimposed upon it, rules at the same time the family's general mode of growth; it intervenes as a limiting factor, independent of the colony's age, and quite distinct, by this fact, from a factor of senescence in the true meaning of this word. However, the colony's initial individuals, the " ciliospores," appear to be characterized by a kind of " physiological potential " greater than that of the main strain's common individuals.

As in all the colonial Vorticellidæ that I have previously studied, they are characterized by large size and by the presence of definite

granulations connected with the secretion of the basic peduncle's inert substance.

During their particular growth, accompanied by a complete changing of the nuclear apparatus, the cells acquire these properties and we can thus show that near the end of the colony's cycle of growth an endomictic cycle exists, closely comparable to that observed in a population of free Infusoria.

But we must remark that, here again, the particular evolution of these "ciliospores" and the endomictic phenomena of which they are the seat, are determined, not by their *age*, but by their place in the colony's plan, just as if this evolution were still connected with the same mechanism of differential division and of nuclear segregation.[5]

I am very glad to be able here to express my thanks to the International Education Board, to my American colleagues who made my residence at Woods Hole so profitable for me, and, very particularly, to Dr. Calkins and Mrs. Harnley, who have helped me in translating this paper.

Summary

1. The first division of the initial macrozooid (or ciliospore) determines the median antero-posterior plane of the colony; the subsequent cleavages of the daughter individuals are brought about according to equally determined schemes, which give the main strain (or axial trunk) and the lateral branches, alternately at right and at left.

2. The individuals constituting the main strain are of a rather large size (axial macrozooids); their cleavage is always accompanied by a differential division giving rise to a new axial macrozooid and a median microzooid.

3. The differential divisions are characterized by an unequal division of the protoplasmic mass, accompanied either by a sensibly equal division of the macronucleus (division supposed to be quantitatively differential), or by the unequal division of the macronucleus in which the larger mass (delicately granular) remains in the larger individual, while the thinner part (often of fibrillar structure) goes to the microzooid (division supposed to be qualitatively differential).

4. The cleavages of the ciliospores and those of the axial macrozooids, I, II, and III are always differential as regards the protoplasm and the nucleus. The cleavages of the macrozooids IV and after give a cytoplasmic differential division and an equal nuclear division; the dif-

[5] Long ago I mentioned an apparently differential division in *Lagenophrys,* in which one of the individuals remained sedentary, while the other migrated and secreted a new shell (1904).

ferential division of the macronucleus is carried back to the cleavage of the corresponding median microzooids.

5. The common microzooids have a limited power of growth and of multiplication.

6. The median individuals having a large macronucleus after the differential division of the median microzooids *D* and progeny begin an active period of growth accompanied or unaccompanied by only one ulterior division: these forms constitute the median macrozooids or "ciliospores."

7. The growth of the ciliospores is accompanied by an important hypertrophy of the macronucleus followed at first by a disintegration, then by a reconstitution through an endomictic process.

8. During the growth of the median macrozooids, some grains of secretion accumulate at the individual's posterior end, then the ciliary crown grows, the ciliospore breaks away, swims freely, then settles down on a substratum and becomes the source of a new colony.

9. The character of the differential divisions on the main strain seems to determine the individual's differentiation of the colony; this differentiation depends not only on the individual's size, but also on its physiological potencies.

10. Independently of the obviously differential divisions, it is shown that the power of growth is divided among the microzooids according to a gradient, so to speak.

11. The unequal power of growth of the various individuals of a colony gives to its whole growth a behavior which approaches the behavior of an organism. This unequal share constitutes for the growth of the whole a limiting factor very unlike a factor of senescence.

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